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Research notes: Aluminum tolerance in soybean germplasm

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Table 3

Physiological characteristics of parental lines

Line	Days to mature	Plant ht, cm	Leaflet* area, cm^2	SLW** mg/cm^2	Chlorophyll** mg/dm^2	P_A** $mgCO_2/dm^2/h$
OX298	121	66	87	5.6	3.8	28
OX610I	124	75	86	5.2	3.7	24
OX615	114	80	72	5.4	4.1	31
OX693	104	57	72	6.8	4.3	31
OX696	128	72	78	4.6	3.6	25
Harcor	125	78	82	5.4	3.9	28
Harosoy 63	122	81	94	5.2	3.7	25
L.S.D. 0.05	-	-	ns	1.19	0.68	5.5
C.V. %	-	-	12.4	9.6	8.1	16.5

*Most recently fully-expanded leaves sampled July 26; 9 per plot in 4 replicates.
**Average of 6-replicate determinations July 28 and August 16, 1978.

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1) Genetic analysis of factors controlling nodulation response in soybeans.

Two of the genes controlling nodulation response in soybeans were tested for linkage associations with genes controlling pubescence color (T) and flower color (W_1), chlorophyll deficiency (y_9) and absence of pubescence (P). The rj_1 gene (Williams and Lynch, 1954) in homozygous recessive condition results in a non-nodulating phenotype with a broad spectrum of Rhizobium japonicum strains. The dominant gene, Rj_4 (Vest and Caldwell, 1972) conditions an ineffective nodulation response when inoculated specifically with R. japonicum strain 61 of the Beltsville Culture Collection.

Genetic stocks (T lines) and Clark rj_1 rj_1 were obtained from the Soybean Genetic Type Collection (Bernard and Weiss, 1973). Crosses were made in the field and F_1 seed were advanced to the F_2 generation in the greenhouse. F_3 seed was produced in the field at Beltsville. F_3 progeny rows derived from individual F_2 plants of rj_1 crosses were evaluated for phenotype in the field at Beltsville. Crosses with Rj_4 were evaluated in plastic growth tray assemblies (Devine and Reisinger, 1978) and inoculated with 7-day-old broth cultures of R. japonicum strain 61. F_2 genotypes were rationalized from F_3 phenotypes. Results of these linkage tests (Table 1) indicate independent assortment of rj_1 and T, rj_1 and W_1, rj_1 and P, and Rj_4 and y_9. A linkage association is apparent between Rj_4 and P in linkage group 2. Reasoning from this

Table 1

Soybean Linkage Test

Genes	a	b	c	d	Sum	%R*	SE	Phase
Clark rj_1 (rj_1 rj_1 T T W_1 W_1) x Hardee (Rj_1 Rj_1 t t w_1 w_1)								
Rj_1 rj_1 T t	110	41	37	10	198	46	5	R
Rj_1 rj_1 W_1 w_1	107	40	36	9	192	44	8	R
Clark rj_1 (rj_1 rj_1 W_1 W_1) x Hill (Rj_1 Rj_1 w_1 w_1)								
Rj_1 rj_1 W_1 w_1	115	40	36	13	204	50	-	R
T135 (rj_4 rj_4 y_9 y_9) x Hill (Rj_4 Rj_4 Y_9 Y_9)								
Rj_4 rj_4 Y_9 y_9	119	37	27	13	196	44	6	C
Hill (Rj_4 Rj_4 p p) x T145 (rj_4 rj_4 P P)								
Rj_4 rj_4 P p	125	66	47	9	244	36	5	R
T145 (Rj_1 Rj_1 P P) x Clark (rj_1 rj_1 p p)								
Rj_1 rj_1 P p	115	33	34	9	191	51	5	C

*Recombination percentages calculated by the product method (Immer and Henderson, 1943).

positive linkage association and the lack of linkage of P with rj_1, we conclude that Rj_4 and rj_1 are not allelic.

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2) Significance of incompatibility reactions of Rhizobium japonicum strains with soybean host genotypes.

Soybeans normally nodulate with Rhizobium japonicum and fix nitrogen in symbiotic association. However, several interactions, under genetic control, have been reported which result in ineffective nodulation or failure of the fixation process despite substantial nodule development. The recessive gene, rj_1, (Williams and Lynch, 1954) in homozygous mode, results in the exclusion from nodulation of a broad spectrum of Rhizobium japonicum strains in soil culture. No evidence of nodule development is visible to the unaided eye. The Rj_2 gene, a dominant factor, reported in the cultivars 'Hardee' and 'CNS' (Caldwell, 1966), results in the formation of cortical proliferations or rudimentary nodules when plants are inoculated with R. japonicum strains of the c1 or 122 serogroup.

The gene Rj_3, also reported in the cultivar Hardee (Vest, 1970), produces an ineffective nodulation reaction specifically with Rhizobium strain 33 of the Beltsville Culture Collection. The Rj_4 gene, reported in the cultivar 'Hill' (Vest and Caldwell, 1972), conditions ineffective nodule development, specifically with R. japonicum strain 61. Another type of incompatible reaction occurs when the cultivar 'Peking' is inoculated with R. japonicum strain 123 (Vest et al., 1972). Nodules are formed in normal frequency and size. However, virtually no nitrogen is fixed. Several other Rhizobium strains exhibit varying degrees of inefficiency in fixation with Peking.

These aberrant reactions have been regarded as interesting but troublesome biological oddities. The literature provides no explanation for their occurrence. Two hypotheses are proposed here.

First, the Rj genes may be "inborn metabolic errors" (analogous to phenylketonuria in man), which arose by mutation in plant breeders' stocks and have not (Devine, 1976) been eliminated from breeders' lines. Second, the incompatible reactions may result from coupling genotypes of the host and microsymbiont which have not coevolved in the same locality. Natural selection would have occurred for mutual compatibility during coevolution in Asia. The reassortment of germplasm of host and microsymbiont occurring with introduction to the New World may have resulted in association of ecotypes alien to each other, resulting in incompatible reactions.

To test these hypotheses, Plant Introductions (PI's) representing several countries and maturity groups are being tested with the Rhizobium strains defining for the Rj reactions. A portion of the results of this survey is presented here.

The Plant Introductions were planted in hills of five seed each in plant growth trays (Devine and Reisinger, 1978), 24 hills per tray. Seed were surface sterilized with 50% ETOH before planting and inoculated with the strain appropriate for definition of the pertinent Rj factor. Plants were evaluated two or three weeks after planting. Approximately 30 PI's were sampled in each of the maturity groups I through VIII. Seven countries are represented in the sample of PI's.

The Rj_2 gene does occur in Asiatic populations, however, at a low frequency in the population sampled (Table 1). The Rj_4 gene occurs with much higher frequency (Table 2). All five PI's from Thailand carry Rj_4 as do four of the five PI's from Indonesia. These results lead to the conclusion that the first hypothesis is not tenable in the case of Rj_2 and Rj_4 and that these

Table 1

Gene frequency of Rj_4 in soybean Plant Introductions from China, Manchuria, Korea, Japan, Taiwan, Thailand and Indonesia

Maturity Group	Origin of Plant Introduction and frequency of lines with Rj_4 phenotype													
	China and Manchuria		Korea		Japan		Taiwan		Thailand		Indonesia		Total	
	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
I	1/16	6	2/4	50	0/10	0	-	-	-	-	-	-	3/30	10
II	5/10	50	0/10	0	3/10	30	-	-	-	-	-	-	8/30	27
III	5/10	50	2/10	20	1/10	10	-	-	-	-	-	-	8/30	27
IV	10/10	100	3/10	30	2/10	20	-	-	-	-	-	-	15/30	50
V	2/10	20	3/10	30	3/10	30	-	-	-	-	-	-	8/30	27
VI	3/10	30	4/9	44	5/10	50	-	-	-	-	-	-	12/29	41
VII	4/11	40	1/6	17	3/11	27	-	-	-	-	-	-	8/28	29
VIII	-	-	-	-	4/13	31	3/5	60	5/5	100	4/5	80	16/28	57

Table 2

Gene frequency of Rj_2 in soybean Plant Introductions from China, Manchuria, Korea, Japan, Taiwan, Thailand and Indonesia

Maturity Group	Origin of Plant Introduction and frequency of lines with Rj_2 phenotype													
	China and Manchuria		Korea		Japan		Taiwan		Thailand		Indonesia		Total	
	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
I	0/16	0	0/4	0	0/10	0	-	-	-	-	-	-	0/30	0
II	0/10	0	0/10	0	0/10	0	-	-	-	-	-	-	0/30	0
III	0/10	0	0/10	0	0/10	0	-	-	-	-	-	-	0/30	0
IV	0/9	0	0/10	0	1/10	10	-	-	-	-	-	-	1/29	3
V	0/10*	0	0/10	0	0/10	0	-	-	-	-	-	-	0/30*	0
VI	1/10	10	0/9	0	0/10	0	-	-	-	-	-	-	1/29	3
VII	1/11	10	0/6	0	0/13	0	-	-	-	-	-	-	1/30	3
VIII	0/2	0	-	-	1/13	1	0/5	0	0/5	0	0/5	0	1/30	3

*Heterogeneity in one line.

genes trace to Asiatic origin rather than recent mutation in U.S. breeding stocks.

Very little information is available on the precise location in Asia from which Rhizobium strains now in the production fields of the United States originated. The frequency with which these genes occur in PI's of the maturity groups and nations sampled suggest that many of the PI's evolved in areas where there was not a significant selection pressure for compatibility with Rhizobium strain 61.

I interpret these results as supporting the concept of coevolution affecting the compatibility of host strain interactions. If the interactions affecting the efficiency of fixation, as seen in the Peking x strain 123 reaction, are analogous to the Rj_2 and Rj_4 phenomenon, it may be postulated that the efficiency of nitrogen fixation in U.S. soybean production may be improved by reassembling the ecotypic associations of soybean germplasm and Rhizobium strains as they evolved in Asia.

The high frequency with which the Rj_4 gene occurs in the PI's and the severity of its effect in restricting nitrogen fixation in association with Rhizobium strain 61, indicates that when breeders are evaluating PI's in soils that are nitrogen deficient, the nature of the Rhizobium strains in the field may profoundly affect the performance of the PI's. In such circumstances, if the breeders' object is to determine the full biological potential of the PI's, they should apply adequate nitrogen fertilizer to their nurseries.

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3) Aluminum tolerance in soybean germplasm.

Aluminum in the soil solution is a severe growth limiting factor in certain acid soils (Foy, 1964; Long and Foy, 1970). This problem is particularly serious in acid subsoils (Adams and Lund, 1966; Foy, 1964) which are difficult to lime. Recent research has centered on selecting those plant cultivars which demonstrate a degree of tolerance to soil aluminum. Research with wheat has shown that differential tolerance to aluminum (Al) is related to the region in which cultivars were bred (Foy et al., 1974). Devine (1976) demonstrated that Al tolerance is a heritable trait in alfalfa and that recurrent selection was an effective breeding method for modifying Al response. The objective of our present research is to identify sources of Al tolerance in soybean germplasm and to develop efficient and precise methods to assay for tolerance. The results reported here reflect a portion of the work concerning several lines of germplasm from Korea chosen for testing because of their origin from areas reputed to have low pH soils.

Plants were evaluated in a hydroponic system using four glass tanks arranged in two replications in a growth room. Forty liters of modified 1/5 Steinburg solution containing 4 ppm Ca was added to each tank (Foy et al., 1967 and 1969). An Al treatment (6 ppm Al as $AlK(SO_4)_2$) was added to one tank in each replication. Solutions in all tanks were aerated continuously and adjusted daily to pH 4.5 with either 1 N HCl or 1 N NaOH as required. The weight of 30 seeds used for germination was determined on a Mettler top-loading balance. The weight per 100 seeds was calculated and is presented in Table 1. Seeds were placed in germination paper and incubated at 26°C for 72 hours in the dark. For each entry, five seedlings per treatment, per replication, were then transferred to the solution culture for 72 additional hours at the same temperature. The plants were given a 16-hour daylength at 2807 lux. Each plant was measured for primary root length (PRL), length from the primary root tip to the most recently emerged secondary root (RPS), and the length of the three longest lateral roots (LRL). In addition, from a comparison of the roots in +Al treatment and -Al treatment, each entry was assigned a visual damage score ranging from 1, least damage, to 5, most damage. The cultivars 'Perry' and 'Chief', known for their respective tolerance and susceptibility to aluminum (Foy et al., 1969), were included as checks.

As a measure of an entry's ability to sustain its normal growth despite Al stress, the ratio +Al/-Al was calculated for each entry in each replication and subjected to an ANOVA. Then, to permit approximate comparisons across a series of tests, the values for the two check cultivars (Perry and Chief) were averaged to obtain a standard value for the test, and the values for the other test lines were compared to this derived standard as a percent of the test standard. A partial summary of the data appears in Table 1 and correlation values are given in Table 2.

Significant differences in Al tolerance among entries were not detected with the visual score. However, with 6 ppm Al, LRL indicated that three entries (635-4, 600-7-2 and 600-4-2) were significantly more tolerant to aluminum than the resistant check cultivar Perry. The LRL ratio (+Al/-Al) also indicated differential tolerance among entries. For example, entry 600-4-2 was significantly more tolerant than the Al-sensitive cultivar Chief. However, no significant differences between Perry and Chief were detected by the parameters measured in this test. Of the four measures for detection of differential Al tolerance, i.e., visual damage score, and the +Al/-Al ratio for PRL,

Table 1

Aluminum toxicity solution culture test no. 12-1978

Entry No.	Weight of 100 seed	Visual damage score[1]	PRL	PRS	LRL			
			% of standard	% of standard	0 Al (cm)	6 ppm Al (cm)	Ratio	% of standard
635-4	20.7	2.5 a*	101 a	108 a	5.5 a	5.1 abc	.99 ab	151 abc
635-6	23.2	3.0 a	105 a	105 a	5.3 ab	4.2 a-f	.85 ab	140 abc
600-7-2	20.0	2.5 a	98 a	102 a	5.0 abc	4.7 a-d	.97 ab	173 ab
635-2	17.6	3.0 a	106 a	96 a	5.9 a	4.5 a-e	.79 ab	148 abc
635-1	23.6	3.5 a	111 a	118 a	4.7 a-d	3.3 b-g	.76 ab	114 c
600-10-1	11.2	3.5 a	95 a	66 a	2.8 d-g	1.5 g	.68 b	117 c
600-8	27.9	3.0 a	107 a	114 a	4.8 a-d	3.3 c-g	.74 ab	126 bc
600-4-2	28.8	3.0 a	107 a	116 a	4.5 a-e	4.7 a-d	1.21 a	194 a
600-6-2	10.9	4.0 a	92 a	78 a	4.4 a-e	3.4 fg	.52 b	99 c
Perry	15.3	3.0 a	--	--	4.1 a-f	2.6 efg	.73 ab	--
Chief	15.3	3.5 a	--	--	4.5 a-e	2.1 g	.54 b	--

[1]Scored 1 to 5: 1 = least damage; 5 = most damage.

*Any two values having a letter in common are not significantly different at the 5% level by the Duncan's multiple range test.

Table 2

Correlation values

Observations	Seed weight	Visual score
Visual score	-.50 NS	--
PRL as % of standard	.82**	-.28 NS
PRL at 0 ppm Al	.35 NS	-.35 NS
PRL at 6 ppm Al	.64*	-.47 NS
PRS as % of standard	.93**	-.51 NS
PRS at 0 ppm Al	-.44 NS	.14 NS
PRS at 6 ppm Al	.57 NS	-.52 NS
LRL as % of standard	.54 NS	-.75*
LRL at 0 ppm Al	.44 NS	-.51 NS
LRL at 6 ppm Al	.64*	-.78*
LRL ratio +Al/-Al	.69*	-.73*

*Significant at the 5% level.
**Significant at the 1% level.
NS = not significant.

PRS and LRL, no significant differences were found for visual damage score or PRL, while PRS and LRL differences were significant. The greatest range in variation was expressed in the +Al/-Al ratio for LRL. For this reason the LRL data are presented in more detail.

To determine the influence of seed reserves on the expression of aluminum tolerance, seed weight was tested for correlation with other measurements (Table 2). Seed weight was positively correlated ($p < .01$) with PRL as percent of standard and with the PRS as percent of standard, but was not significantly correlated with the LRL as percent of standard. This suggests that seed reserves strongly influence the aluminum response of primary root growth. The correlations of seed weight with the PRL, PRS, and LRL at 0 ppm Al were not significant. But, under Al stress at 6 ppm, seed weight was significantly correlated with PRL and LRL and approaches significance with PRS. This suggests that under aluminum stress the influence of seed reserves on growth is greater than in the absence of aluminum stress. Additional correlations were made to determine the influence of the factors measured on the assignment of visual damage scores. The visual damage score was not correlated with seed weight. Nor was visual score correlated with any of the measurements at 0 ppm Al. The visual score was not correlated with PRL or PRS either at 6 ppm Al or as percent of standard, suggesting that these measurements had little or no influence on the assignment of visual ratings. However, the LRL at 6 ppm Al and as percent of standard are negatively correlated with the visual score, indicating the LRL was an important factor influencing this score.

A previous study (Devine, 1976) reported that variation in seed lots of the same cultivar produced at different locations had little effect on aluminum tolerance in comparison with effect of the genotype of the zygote. In that study conducted with adapted U.S. cultivars, seed weight within a cultivar did not vary appreciably. In this study, however, seed weight varied

10.9 to 28.8 g/100 seed, a factor of 2.6. Similar variation would be expected in screening the Soybean Germplasm Collection.

These results indicate that seed weight exerts an influence on early seedling expression of Al tolerance and caution should be used in imputing long term physiological tolerance to lines expressing tolerance at this stage.

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